PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/569, A61K 39/118 // C07K 3/02

A1

(11) International Publication Number: WO 90/13032

(43) International Publication Date: 1 November 1990 (01.11.90)

(21) International Application Number: PCT/GB90/00568

(22) International Filing Date: 12 April 1990 (12.04.90)

(30) Priority data:

8908548.4	14 April 1989 (14.04.89)	GB
9004845.5	5 March 1990 (05.03.90)	GB

(71) Applicant *(for AU only)*: UNILEVER PLC [GB/GB]; Unilever House, P.O. Box 68, Blackfriars, London EC4P 4BQ (GB).

(71) Applicant *(for JP only)*: UNILEVER NV [NL/NL]; Burgemeester 's Jacobplein 1, P.O. Box 760, NL-3000 DK Rotterdam (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)* : DAVIDSON, Ian, William [GB/GB]; "Braeside", Springhill, Little Staughton, Bedfordshire MK44 2BS (GB). SHEARD, Paul [GB/GB]; 15 Silver Street, Broughton, Kettering, Northants NN14 1PA (GB).

(74) Agent: BUTLER, David, John; Patent Division, Unilever PLC, Unilever House, P.O. Box 68, Blackfriars, London EC4 4BQ (GB).

(81) Designated States: AU, JP, US.

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: EXTRACTION PROCEDURE

(57) Abstract

Enhanced extraction of solubilised antigens is obtained from bacteria such as *Chlamydia* and *Neisseria* by the use of a buffer containing a zwitterionic surface active agent, especially CHAPS or CHAPSO, in the absence of divalent cations. The extraction is conducted at elevated temperature, and provides a sample useful in assays for the presence of the bacteria.

AN2

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CM	Cameroon
DE	Germany, Federal Republic of
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GR	Greece
HU	Hungary
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
RO	Romania
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

EXTRACTION PROCEDURE

The present invention relates to procedures for extracting antigenic material in solubilised form from cellular biological materials such as bacteria. The solubilised antigenic material can be used thereafter in assay procedures to determine the presence or identity of the cellular material.

The use of surface active agents in extraction media at elevated temperature has been proposed. Examples are given in EP 167395 and EP 183383, both of which relate to extraction procedures especially applicable to species of Chlamydia.

EP 183383 says that it is beneficial to have divalent cations, specifically magnesium and zinc, present during the heating stage of an extraction procedure.

However, in complete contrast, we have found that a better extraction of antigenic material can be obtained in the absence of divalent cations.

The invention provides a procedure for extracting solubilised antigenic material from cellular biological material, such as bacteria, wherein the cellular material is treated with an aqueous solution of a surface active agent, the solution being substantially free from divalent cations. Preferably the surface active agent is zwitterionic. More preferably, the surface active agent is 3-(3-chlolamidopropyl)dimethylammonio-1-propanesulfonate (conveniently known as CHAPS) or 3-(3-chlolamidopropyl)dimethylammonio-2-hydroxyl-1-propanesulfonate (conveniently known as CHAPSO), or mixtures thereof.

In particular, we have found that an especially effective extraction of lipopolysaccharide antigen from Chlamydia species such as Chlamydia trachomitis, C. psittaci and C. twar, is achieved if the extraction is performed using an aqueous solution of a zwitterionic surface active agent, especially CHAPS and/or CHAPSO.

We have also found that an especially effective extraction of a proteinaceous antigen from Neisseria gonorrhoeae is achieved if the extraction is performed using an aqueous solution of a zwitterionic surface active agent, especially CHAPS and/or CHAPSO.

Preferably, the extraction is conducted at elevated temperature, for example in excess of about 50°C, for a period of time sufficient to solubilise the antigenic material. More preferably, the extraction temperature is at least about 60°C. In general, the extraction temperature need not be greater than about 100°C, and is preferably not greater than about 90°C. Ideally, the extraction temperature is about 80°C. The stage of the extraction conducted at such elevated temperature should generally last for at least about 5 minutes.

Preferably, the quantity of surface active agent in the aqueous extraction medium is at least about 0.1% by weight. Preferably the quantity of surface active agent is not greater than about 2%, and more preferably not greater than about 1% by weight.

The pH of the extraction medium should generally be in the range of about 7.5 to about 9.

As stated above, the extraction medium should be substantially free from divalent cations. In particular, zinc and magnesium ions should not be present in any appreciable quantity. This can be achieved by using ion-free water and other components when preparing the extraction medium. Alternatively, or in addition, chelating agents such as EDTA, EGTA or DPTA can be incorporated in the extraction medium to remove, in effect, any divalent cations that may be present. If the performance of the subsequent assay applied to the extracted antigen solution may be adversely influenced by ionic strength, it is preferable that the chelating agent is used in the free acid form, rather than as a water-soluble salt such as its sodium salt; this appears to be an important consideration in the case of extraction from Neisseria. We believe that the effective absence of divalent cations enhances disruption of epithelial cellular material which in turn enhances extraction of any bacteria such as Chlamydia which is an intra-cellular parasite.

In a typical extraction procedure according to the invention, a biological sample obtained from a patient suspected of carrying a Chlamydia infection, for example, is contacted with an extraction medium containing the CHAPS and/or CHAPSO. Appropriate samples can take the form, for example, of genital, rectal or ocular swabs, or

centrifugal pellets from liquids such as early morning urine. Extraction, for example at 80°C for 10 minutes, is followed by a brief period, for example 5 minutes, during which the extraction medium is allowed to cool. Thereafter the extraction medium can be separated from solid matter, for example by removal of the swab and filtration of the solution to provide a sample liquid containing any extracted antigen ready for use in any suitable assay procedure. The subsequent assay can involve any conventional assay technique, such as radioimmunoassay or enzyme-linked immunoassay. The extracted sample is ideal for use in an immunochromatographic assay procedure such as described and claimed in GB 2204398 A, especially using a nitrocellulose solid phase and an antibody reagent labelled with a direct particulate label such as coloured latex particles. The use of the CHAPS and/or CHAPSO enhances the sensitivity of a Chlamydia assay which involves anti-lipopolysaccharide antigen antibodies as specific binding reagents.

Example 1

An extraction procedure for Chlamydia trachomatis can be performed as follows. This provides an extract suitable for use in an immunoassay.

The extraction procedure utilises:

a) Disposable, flexible plastics "test tubes" each capable of holding a volume (eg. 5 ml) of liquid and of accommodating the end of a conventional sampling swab.

b) A heating block in which such tubes can be inserted to permit the contents of the tube to be heated to a

temperature in the range 50 - 100°C and held at that temperature for at least a number of minutes.

c) Means for filtering the liquid contents of the tube at the end of the extraction procedure. Conveniently this can take the form of a filter plug incorporated in a perforated stopper with which the tube can be closed and through which the liquid contents can be expelled.

d) An extraction buffer having the following formulation:

0.1 M TRIS pH 8.5 containing

0.85% Sodium chloride
0.25% CHAPSO
1% Bovine serum albumin
5m M EDTA

To conduct the extraction, a pre-determined quantity (for example 600 µl) of extraction buffer is placed in a tube. A genital swab from a patient suspected of carrying a Chlamydia infection is placed in the tube, and the tube and contents are then incubated in the heating block at a temperature of approximately 80°C for 10 minutes. The tube is removed from the heating block and allowed to cool for 5 minutes. The swab is lifted out of the extraction buffer and, before the swab is removed completely from the tube, the sides of the tube are pressed in gently by hand to squeeze liquid from the swab. The swab can then be removed completely from the tube and discarded. A perforated stopper containing a filter plug is inserted in the top of the tube, and the liquid contents of the tube can be expelled therethrough to provide an essentially clear liquid sample containing any extracted Chlamydia lipopolysaccharide antigen for

use in a subsequent assay. If desired, one or more assay reagents, such as labelled antibodies, can be added to the contents of the tube before the filter/stopper is applied to the tube.

Example 2

The same extraction procedure as Example 1 is followed. However, the extraction buffer has the formulation:

0.1 M TRIS pH 8.5 containing:

1.25%	Sodium Chloride
0.25%	CHAPSO
1%	Bovine Serum Albumin
5m M	EDTA
10m M	L. Ascorbic Acid.

An increased level of sodium chloride helps to prevent non-specific binding, which may occur in the subsequent immunoassay. The presence of ascorbic acid (preferably L form) has been found to increase the storage life of the extraction buffer, where it acts as a stabilizer, when the buffer is subjected to adverse conditions (eg. increased temperature during distribution, or long-term refrigerated storage).

Claims

1. A procedure for extracting solubilised antigenic material from cellular biological material, wherein the cellular material is treated with an aqueous solution of a zwitterionic surface active agent, the solution being essentially free from divalent cations.

2. A procedure for extracting solubilised antigenic material from cellular biological material, wherein the cellular material is treated with an aqueous solution of CHAPS and/or CHAPSO.

3. A procedure according to claim 1 or claim 2, wherein the cellular material comprises bacteria.

4. A procedure according to claim 3, wherein the bacteria comprise Chlamydia trachomatis.

5. A procedure for extracting solubilised lipopolysaccharide antigen from bacterial cells, wherein the cells are incubated in an aqueous solution of CHAPS and/or CHAPSO at a temperature in excess of 50°C for a period of time sufficient to solubilise the antigen.

6. A procedure for extracting solubilised lipopolysaccharide antigen from Chlamydia trachomatis, wherein Chlamydia trachomatis cells are incubated in an aqueous solution containing from 0.1 to 0.3% by weight of surface active agent selected from the group consisting of CHAPS, CHAPSO and mixtures thereof, the solution being essentially free from divalent cations, and the incubation including a stage conducted at a temperature in the range 60 - 100°C for a period of at least 5 minutes.

7. An aqueous extraction buffer for solubilising antigenic material in bacteria and the like, containing an effective amount of CHAPS and/or CHAPSO.

8. A buffer according to claim 7, essentially free from divalent cations.

9. A buffer according to claim 7 or claim 8, containing ascorbic acid.

10. Use of a buffer according to any one of claims 7 to 9, to enhance the extraction of solubilised antigenic material from bacteria such as Chlamydia and the like.

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/569 ; A61K39/118 ; //C07K3/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N ; A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	BIOSIS, vol 83, 1987, abstr.no.83047832 W.Stuer et al., "Purification of extracellular lipase from Pseudomonas-Aeruginosa" & J.Bacteriol.,vol.168,no.3,1986,pp.1070-1074 ---	1-3, 7, 8, 10
A	US,A,4707543 (W.D.ZOLLINGER ET AL.) 17 November 1987 see column 3, lines 25 - 30 ---	5
X	BIOSIS,vol.87,1989,abstr.no.87119085 P.Domenico et al.,"Quantitative extraction and purification of exopolysaccharides from Klebsiella Pneumoniae" & J.Microbiol.Methods,vol.9,no.3,1989,pp.211-20 ---	1, 3
X		1, 3, 5, 8

-/--

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20 JULY 1990	24 AUG 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	DE KOK A.J.

3

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	BIOSIS,VOL.86,1988,ABSTR.NO.86104274 M.O.Labeta et al.,"Solubilization effect of Nonidet P-40, Triton X-100 and CHAPS in the detection of MHC-like glycoproteins" & J.Immunol.Methods,vol.112,no.1,1988,pp.133-38	1, 2, 7, 10
A	EP,A,183383 (IQ(BIO)L.D.) 04 June 1986 see column 1, line 1 - column 4, line 5 (cited in the application)	1-6
A	DD,A,242716 (AKADEMIE DER WISSENSCHAFTEN DER DDR) 11 February 1987 see the whole document	1-3, 7
P,X	EP,A,325045 (SYNTEX (USA) INC.) 26 July 1989 see page 5, line 42 - page 6, line 24 see page 11, lines 6 - 8	1-5, 7, 10
P,X	EP,A,334278 (W.BREDT ET AL.) 27 September 1989 see page 3, lines 46 - 51 see page 5, lines 39 - 50	1-3, 7, 8, 10
P,X	WO,A,8908262 (KALLESTAD DIAGNOSTICS INC.) 08 September 1989 see page 3, lines 2 - 29 see page 9, lines 20 - 36	1-8, 10

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9000568
SA 36102

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 20/07/90

Patent document cited in search report	Publication ste	Patent family member(s)	Publication date
US-A-4707543	17-11-87	None	
EP-A-183383	04-06-86	US-A- 4830960	16-05-89
DD-A-242716		None	
EP-A-325045	26-07-89	None	
EP-A-334278	27-09-89	DE-A- 3809796 AU-A- 3158189	05-10-89 28-09-89
WO-A-8908262	08-09-89	US-A- 4916057	10-04-90